

The relationship of gamma-1A globulin and reagin in cord sera

Mathea Allansmith, M.D., and Donald N. Buell, B.A., Stanford, Calif.

Recent reports¹⁻³ have suggested that reaginic antibody is a gamma-1A (beta-2A) globulin. Reagin does not cross the placenta according to the report of Sherman⁴ who examined the sera of 12 atopic mothers and the cord sera of their infants. Gamma-1A also does not cross the placenta and is first seen in the serum of the newborn at 3 to 4 weeks of age.^{5, 6} The exclusion of both reaginic activity and gamma-1A globulin (determined in separate studies) from the fetal circulation when so few other serum factors are lacking constitutes a strong presumption that reagin is a gamma-1A globulin. This study was performed to obtain factual evidence for this presumption. The maternal-cord relationship of gamma-1A globulin was studied in a group of atopic and of non-atopic mothers. In addition, the occurrence in cord serum of the other two antibody globulins, gamma-2 and gamma-1M was examined.

MATERIALS AND METHODS

Sera

Eighty-four paired maternal and cord sera were obtained over a 3-year period from mothers on the obstetric service of the Palo Alto-Stanford Hospital. Twenty-six mothers gave a positive wheal-and-flare response to an intradermal injection of 0.025 ml. of a 1:1,000 by weight dilution of mixed grass pollen antigen and were designated as atopic. The 58 showing no response were placed in the normal category. Maternal blood was drawn from two months ante partum to three days post partum; most samples were taken post partum.

From the Department of Medical Microbiology, Stanford University.

This investigation was supported in part by the United States Public Health Service Postdoctoral Fellowship EPD-11,682-C2, American Cancer Society Grant E320, and Public Health Service Training Grant 5 T1 AI 82 of the National Institute of Allergy and Infectious Diseases.

Received for publication Jan. 13, 1964.

Cord blood was obtained following delivery of the placenta and a sample was obtained from one baby on the eighth postnatal day also. A few of the cord samples were refrigerated for one to four days before centrifugation; otherwise all sera were removed within 24 hours and stored frozen. The 26 atopic and 13 of the normal sera had been previously thawed and refrozen.

Immunoelectrophoresis

The method of Hirschfeld⁷ was used. Slides were prepared using 1 per cent Noble agar in Spinco B-2 veronal buffer, pH 8.6. Groups of six slides were run at 150 volts for 120 minutes.

Antisera

Commercial goat anti-whole human serum (WHS), goat anti-gamma-1A myeloma protein, and goat anti-gamma-1M globulin sera were obtained from Hyland Laboratories, Los Angeles, Calif. Rabbit anti-human gamma-2 globulin was obtained from Antibodies Incorporated, Davis, Calif. The anti-gamma-2, gamma-1A and gamma-1M sera were found by immunoelectrophoresis to develop single strong specific precipitin lines with whole human serum. The anti-gamma-2 detected this globulin in a 1:500 dilution of normal human serum; the anti-gamma-1M detected its antigen in a 1:32 dilution, and the anti-gamma-1A detected its globulin in a 1:64 dilution.

Thirty-five mother-cord serum pairs were compared by immunoelectrophoresis developed with anti-gamma-1A globulin. An additional 49 pairs (including some of the atopic sera) were compared with the use of a mixture of gamma-1A and gamma-1M antisera. Identities of the observed precipitin lines were confirmed by comparison with those found with goat anti-WHS.

Five atopic maternal-cord pairs and four normal maternal-cord pairs were examined by a single diffusion in plates of the various antibodies incorporated in agar* (Fig. 2). Serum, 0.004 ml., was placed in a well made with a 13 gauge needle from which the bevel had been removed. Antibody in the agar combines with its specific serum component to form a precipitin ring the size and density of which depend upon the quantity of antigen present. The least amount of antigen detectable by the antiserum in the agar was 10 mg. per 100 ml. for the anti-gamma-2 and anti-gamma-1M plates, and 15 mg. per 100 ml. for the anti-gamma-1A plates.

Prausnitz-Küstner tests

Standard P-K tests^{2, 4} were performed on all atopic and normal maternal and cord sera using mixed grass pollen antigens 1:1,000. The recipients for these tests were nonatopic young male adults.

RESULTS

Immunoelectrophoretic observations are summarized in Table I, and the total results of these and single diffusion antibody plate data are summarized in Table II.

*"Immunoplates" from Hyland Laboratories, Los Angeles, Calif.

Table I. *Immunoelectrophoretic demonstration of immunoglobulins in maternal and cord sera*

	γ_2	γ_{1A}	γ_{1M}
<i>Atopic</i>			
Mother	2/2*	26/26	2/2
Cord	2/2	1/26	1†/2
<i>Normal</i>			
Mother	4/4	58/58	47/47
Cord	4/4	1/58	47/47
<i>Total</i>			
Mother	6/6	84/84	49/49
Cord	6/6	2/84	48/49

*Numerator, number positive.
Denominator, number examined.
†Immunoelectrophoretic pattern inadvertently discarded at 24 hours. Same serum positive in γ_{1M} antibody plate. See Fig. 2.

Table II. *Total immunoglobulin findings in maternal and cord sera (immunoelectrophoretic and single diffusion data)*

	γ_2	γ_{1A}	γ_{1M}
Mother	15/15*	84/84	49/49
Cord	15/15	2/84	49/49

*Numerator, number positive.
Denominator, number examined.

Table III. *P-K Tests with maternal and cord sera*

	Number	Positive P-K	Presence of γ_{1A} -globulin
<i>Atopic</i>			
Mother	26	13	26
Cord	26	1*	1*
<i>Normal</i>			
Mother	58	0	58
Cord	58	0	1

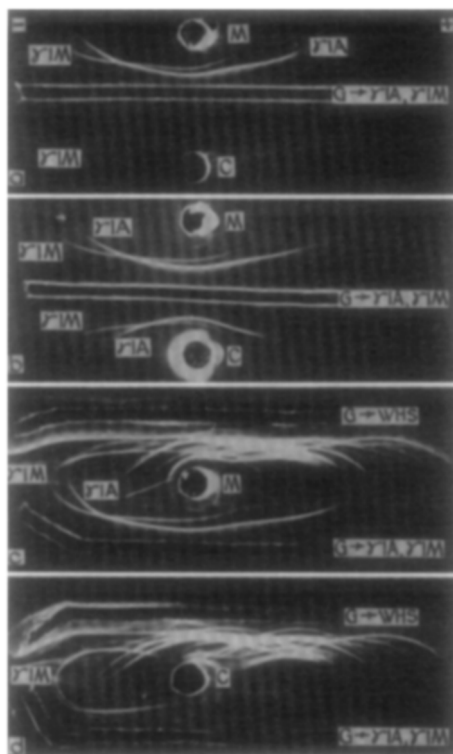
*Same serum.

Gamma-2

In six instances where anti-WHS serum was used to develop maternal and cord immunoelectrophoretic patterns, gamma-2 globulin precipitin lines were found. The plates with rabbit anti-gamma-2 incorporated into the agar also showed approximately equal amounts of gamma-2 globulin in maternal and cord sera (Fig. 2, gamma-2) as judged by the diameter and intensity of precipitin rings produced.

Gamma-1M

All 49 maternal sera examined, including those from atopic mothers, showed strong gamma-1M precipitin lines. Gamma-1M was also detectable in cord sera,

**Fig. 1**

Immunoelectrophoretic identification of γ_{1A} and γ_{1M} globulins in maternal (*M*) and cord (*C*) sera. ($G \rightarrow$ WHS = goat anti-whole human serum. $G \rightarrow \gamma_{1A}$, γ_{1M} = mixture of goat anti γ_{1A} -globulin and goat anti γ_{1M} -globulin.) *a*, Typical pattern. Note absence of γ_{1A} and presence of faint γ_{1M} line in cord serum. *b*, One of two instances where strong γ_{1A} and γ_{1M} lines appeared in cord serum. *c*, Maternal serum from (*a*) developed to show identity of γ_{1A} and γ_{1M} precipitin lines. *d*, Cord serum from (*a*) developed as (*c*) confirming identity of γ_{1M} in cord serum.

but the lines were usually much fainter than those of maternal sera (Fig. 1, *a*). The faintest lines required 24 to 48 hours to become visible, but others were readily seen at 24 hours. Gamma-1M globulin was found in 48 of 49 cord sera examined by immunoelectrophoresis. The one exception, in the atopic group, was inadvertently discarded after 24 hours and thus not observed for a late-appearing faint precipitin line. This cord serum did contain gamma-1M globulin as shown by its precipitation with goat anti-gamma-1M in the agar diffusion plate (Fig. 2, γ_{1M}).

Gamma-1A

All 84 maternal sera examined for gamma-1A globulin showed heavy precipitin lines with no differences noted between the normal and atopic groups. However, only two of 84 cord sera showed gamma-1A precipitin lines by these methods (Figs. 1, *a*, 1, *b*) (Fig. 2, γ_{1A}). It is interesting that these two cord sera also showed strong gamma-1M precipitin lines. Fortunately, the mother

in one of these instances was atopic and showed a 1:100 P-K titer to mixed grass. The corresponding cord serum showed a 1:10 P-K titer (Table III). This baby was negative to direct intradermal test at 30 hours after delivery. The mother's serum produced a positive P-K response in her baby at that time, indicating the capacity of its skin to respond to the appropriate reagin-antigen complex. The possibility that the infant himself made the reagin and gamma-1A globulin is unlikely since serum obtained from this infant at 8 days showed no P-K activity and no gamma-1A globulin. The gamma-1M precipitin line was fainter in the 8 day serum than in the cord sample.

The results of P-K testing of maternal and cord sera are given in Table III. The sera from 13 of the mothers gave positive tests to mixed grass, although all 26 had spring hay fever. As noted above, one cord serum gave a positive P-K test.

None of the 116 sera from the 58 normal mother-cord pairs showed a positive P-K test, as expected.

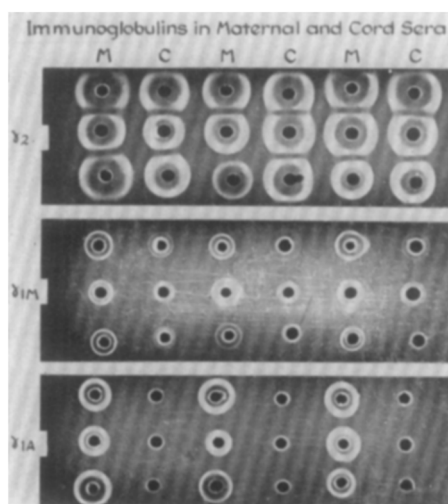


Fig. 2

Nine maternal (*M*) and cord (*C*) sera compared in plates in which antibody has been incorporated into the agar. Gamma-2 contains rabbit anti- γ_2 , γ_{1M} contains goat anti- γ_{1M} , γ_{1A} contains goat anti- γ_{1A} . Maternal and cord sera were placed in adjacent wells. Each pair occupies the same position in all 3 plates. The halo associated with the cup itself is light reflection.

DISCUSSION

In 84 instances in which maternal-cord serum pairs were examined for the various immunoglobulins, both gamma-2 and gamma-1M were found in corresponding samples in all cases studied (15 pairs for gamma-2; 49 pairs for gamma-1M). In contrast, while gamma-1A appeared in all mothers' sera (84), it was found in only two of the 84 cord samples. In these two exceptions, both gamma-1A and gamma-1M globulins were present in relatively large amounts

as indicated by strong precipitin lines. It seems likely that this resulted from admixture of maternal blood near the time of delivery of the placenta. In one of these instances the labor was described as very prolonged and unusually painful. This mother was atopic, with a P-K titer of 1:100. The cord blood titer was 1:10, suggesting that a tenfold dilution of maternal in cord blood occurred. A direct intradermal test at 30 hours was negative in this baby, indicating that the amount of maternal reagin passing into the baby was insufficient to sensitize it. Since the mother's serum gave a positive P-K in this baby at the same time, the skin was capable of responding. The ability of newborn skin to react in a P-K test was not peculiar to this infant, since four other neonates gave positive P-K responses to sensitization with their mothers' sera as well as samples from other atopic subjects, and negative responses to normal serum. Two babies of normal mothers gave the same P-K responses as did babies of atopic mothers.

At eight days, serum from the infant whose cord blood had contained reagin showed absence of this and of gamma-1A globulin. The intensity of the gamma-1M line in the eight-day sample was reduced from that in the cord. The gamma-1M globulin was probably present in the fetal serum in the usual small amount with an aliquot of maternal blood containing gamma-1A and additional gamma-1M entering the cord only at or near the time of placental expulsion. The amount of reagin reaching and being distributed throughout the baby was insufficient to give a positive direct skin test in him.

It is noteworthy that in the atopic group the one instance of P-K activity seen in cord serum was associated with the only case in which gamma-1A globulin was also present. The sera of 12 other atopic mothers were positive in P-K tests, but the cord blood of their infants showed neither a positive test nor gamma-1A globulin. This correlation strongly suggests that gamma-1A globulin is the carrier of allergic skin-sensitizing antibody activity.

In this study, as in another,⁹ gamma-1M was consistently demonstrated in cord sera (49 of 49) though in amounts much less than in corresponding maternal sera. This is not evidence of passage from the mother to the fetus, for neonates and prematures are capable of producing macroglobulin antibodies,¹⁰ and Mellors and associates¹¹ have recently identified gamma-1M globulin in the thymus cells of premature twins born at 30 weeks of gestation. The fetus thus appears capable of producing the trace levels of gamma-1M globulin found in the cord. The alternative explanation of partial placental transmission of maternal gamma-1M seems less likely as cord serum does not show gamma-1M antibody (macroglobulin isoagglutinins) activity even at 1/10 to 1/20 the maternal titer.¹²

The macroglobulin gamma-1M was originally thought to be absent from cord serum. This finding along with the absence of reagin in cord serum suggested that the two activities were associated.⁸ The error of this conclusion has been shown by a number of studies which have separated them, including analytical ultracentrifugation,¹³ preparatory gradient ultracentrifugation,¹⁴ Sephadex G-200 column chromatography,² selective P-K inhibition,³ and selective globulin precipitation.² These studies show that though neither gamma-1M

globulin antibody activity nor reagin passes through the placenta reagin is not associated with gamma-1M globulin.

SUMMARY

1. Gamma-1A globulin and P-K activity were absent in 25 of 26 cord sera of babies born to atopic mothers. In the one exception, both appeared in the cord serum and were absent on rebleeding the infant at eight days. The 26 mothers were skin-test positive to mixed grass and had spring hay fever, 13 had positive P-K tests including the mother of the infant with cord serum reagin, and all had gamma-1A in their blood. Gamma-1A globulin was present in all of 58 sera from normal mothers and was present in 1/58 of the respective cord sera giving an over-all incidence for the presence of gamma-1A in the cord of 2/84. All normal maternal and cord sera were devoid of P-K activity.

2. Gamma-1M globulin was detected in all of 49 maternal-cord serum pairs (part of the atopic group included). The cord gamma-1M concentration appeared to be in 1/10 to 1/20 that of the maternal level.

3. Gamma-2 globulin was detected in 15/15 pairs and appeared to be present in approximately equal concentrations in maternal and cord sera.

REFERENCES

1. Heremans, J. F., and Vaerman, J. P.: β_{2A} -Globulin as a Possible Carrier of Allergic Reaginic Activity, *Nature* **193**: 1091, 1962.
2. Fireman, P., Vannier, W. E., and Goodman, H. C.: The Association of Skin-Sensitizing Antibody With β_{2A} -Globulins in Sera From Ragweed Sensitive Patients, *J. Exper. Med.* **117**: 603, 1963.
3. Ishizaka, K., Ishizaka, T., and Hornbrook, M.: Blocking Sensitization With Reagin by β_{2A} -Globulin, *J. ALLERGY* **34**: 395, 1963.
4. Sherman, W. B., Hampton, S. F., and Cooke, R. A.: The Placental Transmission of Antibodies in the Skin Sensitive Type of Human Allergy, *J. Exper. Med.* **72**: 611, 1940.
5. Hitzig, W. H.: Die physiologische Entveicklung der "Immunglobuline" (Gamma-und-Beta₂-Globulin), *Helvet. paediat. acta* **12**: 596, 1957.
6. West, C. D., Hong, R., and Holland, N. H.: Immunoglobulin Levels From the New-born Period to Adulthood and in Immunoglobulin Deficiency States, *J. Clin. Invest.* **41**: 2054, 1962.
7. Hirschfeld, J.: Immuno-electrophoresis: Procedure and Application to the Study of Group-Specific Variations in Sera, *Science Tools* **7**: 18, 1960.
8. Raffel, S.: *Immunity*, ed. 2, New York, 1961, Appleton-Century-Crofts, Inc., Chap. 13, p. 304.
9. Franklin, F. C., and Kunkel, H. G.: Comparative Levels of High Molecular Weight (19S) Gamma Globulin in Maternal and Umbilical Cord Sera, *J. Lab. & Clin. Med.* **52**: 724, 1958.
10. Smith, R. T.: Response to Active Immunization of Human Infants During the Neonatal Period, in Wolstenholme, C. E. W., and O'Connor, M., editors: *Symposium on Cellular Aspects of Immunity*. Ciba Foundation Symposium, Boston, 1960, Little, Brown and Company, pp. 348-368.
11. Mellors, R., and Korngold, L.: The Cellular Origin of Human Immunoglobulins (γ_2 , γ_{1M} , γ_{1A}), *J. Exper. Med.* **118**: 387, 1963.
12. Kochwa, S., Rosenfield, R. E., Tallal, L., and Wasserman, L. R.: Isogglutinins Associated With ABO Erythroblastosis, *J. Clin. Invest.* **40**: 874, 1961.
13. Heimlich, E. M., Vannier, W. E., and Campbell, D. H.: Sedimentation Studies of Skin-Sensitizing Antibody, *J. ALLERGY*, **31**: 364, 1960.
14. Allansmith, M.: Unpublished data.